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(54) Title: ANTI REPRODUCTIVE HORMONE

(57) Abstract

Immunoneutering of mammalian animals is achieved by vaccinating the animals with a composition comprising an immunogenic protein such as bovine serum albumin, conjugated with a peptide selected from the group comprising any continuous 5, 6 or 7 amino-acid fragment of the decapeptide pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2 (being lutenising hormone releasing hormone) and a suitable immunoadjuvant such as diethylaminoethyl dextran, or emulsions of diethylaminoethyl dextran in a mineral oil. The peptide fragments containing the pGlu from the N terminal or the Gly-NH_2 from the C terminal of the decapeptide are preferred for use in such immunogenic compositions.

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ANTI REPRODUCTIVE HORMONE

TECHNICAL FIELD

This invention deals with immunological methods that may be used to block or suppress sexual activity in animals and with vaccines that induce specific kinds of hormone autoimmunity to achieve the aims of the invention.

BACKGROUND ART

A large body of knowledge exists concerning the endocrine factors that act together to regulate the sexual activity of both male and female mammals. At one level of regulation, the decapeptide comprising mammalian luteinizing hormone releasing hormone (hereafter designated LHRH(1-10)) is secreted from the hypothalamus of the brain and acts at the pituitary gland to cause the pituitary gland in turn to secrete the well known gonadotrophic hormones, luteinizing hormone (LH) and/or follicle stimulating hormone (FSH). The gonadotrophins then exert important biological actions at the level of the gonads to cause these glands to secrete oestrogenic hormone in the female particularly, and androgenic hormones in the male particularly.

Finally, the oestrogenic and the androgenic hormones participate in feedback mechanisms on the brain to regulate their own secretion so that only those concentrations of these hormones that are allowed by homeostasis occur in the circulation of animals. Both oestrogens and androgens are known to act biologically at the level of the central nervous system to regulate sexual behaviour in both male and female animals. They may also act at peripheral sites to affect such important processes as growth rate and nutrient partitioning between muscle and adipose tissue. The important relevant consideration in this complex series of biological processes is that the secretion of gonadal oestrogens or androgens is ultimately under the control of LHRH (1-10).

It is well known that when animals are made immune to LHRH (1-10), the sequenc of endocrine vents that causes the gonads to secrete either oestrogen or androgen is blocked and, in consequence, sexual activity is blocked and sex-hormone-dependent physiological process are either blocked or suppressed.

This occurs because LHRH (1-10)-specific antibody provoked in the immune response binds to endogenous LHRH (1-10) and prevents or inhibits the decapeptide from binding to its receptors and expressing its biological activity. The term "immunocastrate" has been coined to describe the physiological state of LHRH-immune animals because of the similarity with surgically castrate animals residing in the inability of the former to express sexual behaviour. A better term might be "immunoneutered" to take account of the fact that LHRH (1-10) specific antibody will block sexual activity in both male and female mammals.

Various attempts have been made to develop commercial anti-LHRH (1-10) vaccines with the objective of achieving an immunological method of controlling sexual activity in animals.

In the prior art, such anti LHRH (1-10) vaccines have been prepared by chemically conjugating LHRH (1-10) to an immunogenic protein and formulating the immunogenic conjugate so formed together with an immunoadjuvant. Nevertheless for numerous perceived applications of such a vaccine the cost of synthesising the LHRH (1-10) decapeptide has been a seriously limiting factor partly explaining the slow pace of technological development in this area. This invention is concerned with discoveries that substantially simplify the chemical nature of the immunogenic conjugate necessary to stimulate an anti LHRH (1-10) antibody response and that enhance the scope for anti LHRH (1-10) vaccines to be applied to the

immunoneutering of animals.

DISCLOSURE OF THE INVENTION

The present invention consists in a composition for use in the immunoneutering of mammalian animals comprising a peptide conjugated with an immunogenic protein, the composition being characterised in that the peptide includes at its free end a sequence selected from the group comprising any contiguous 5, 6 or 7 amino acid fragment of the decapeptide pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2.

In another aspect the present invention consists in a vaccine for immunoneutering mammalian animals comprising a composition according to this invention together with an immunoadjuvant.

In a still further aspect the present invention consists in a method for the immunoneutering of male or female mammalian animals comprising administering to the animal an effective amount of a vaccine according to the present invention.

For the purpose of this invention LHRH (1-10) is defined to be the decapeptide represented by the amino acid sequence:

pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2

1 2 3 4 5 6 7 8 9 10

Wherein the symbols, according to convention, represent the amino acids as follows: pGlu (pyroglutamic acid), His (histidine), Trp (tryptophan), Ser (serine), Tyr (tyrosine), Gly (glycine), Leu (leucine), Arg (arginine), Pro (proline). Likewise, according to convention, pGlu is located at the N terminus of the decapeptide and Gly-HN_2 is located at the C terminus, it being noted that at the N terminus the cyclic form of the pyroglutamic acid means that there is no free amino function at that terminus and at the C terminus, amidation of the glycine moiety means that there is no free carboxyl function at that terminus.

Except for glycine which is not optically active, all amino acids are of the L configuration. The numbering of the amino acids in the decapeptide as shown above is as commonly used in the art, that is, pGlu, at the N terminus is designated the first amino acid residue with subsequent numbering proceeding sequentially toward the C terminus.

Throughout the description of this invention the symbol, LHRH (x-y), where x and y are numbers chosen from 1 to 10, is used to represent the parent LHRH decapeptide or a designated LHRH fragment peptide. The values of x and y define the first and last amino acids in the peptide sequence. Thus, for example the peptide LHRH (1-7) is composed of the amino acids numbered 1 to 7 in the above formula.

The peptide fragments that can be used to form the novel peptide: protein conjugates of the invention and that can thus become haptens in such conjugates are comprised of any five or any six or any seven amino acids linked as they occur contiguously in endogenous mammalian LHRH (1-10) with or without an additional amino acid or sequence of amino acids acting to link the peptide to the immunogenic protein.

Two kinds of knowledge in the prior art bear on the present invention. On one hand it was known from in vitro measurements that antibodies raised against small chemically synthesised peptides are capable of binding to large protein molecules where such peptide sequences occur at conformationally mobile sites. On the other hand, in the particular case of LHRH (1-10), itself a relatively small hormonal peptide rather than a protein, there are numerous examples of penta, hexa or heptapeptide fragment sequences of that molecule that in vitro will not bind or will only poorly bind to antibodies specifically raised against LHRH (1-10), see for example the following:

(a) Arimura et al, Acta Endocrinologica, 1975, 78,

222-231;

(b) Nett et al, Journal of Clinical Endocrinology and Metabolism, 1973, Vol. 36, No. 5, p.880-885;

(c) Koch et al, Biochemical and Biophysical Research Communications, 1973, Vol. 55, No. 3, p. 616-622;

(d) Jeffcoate et al, Immunochemistry 1974, 11, 75-77;

(e) Pique et al, Immunochemistry 1978, 15, 55-60.

This fact suggests that such peptides do not frequently adopt the conformation of LHRH (1-10) that provoked the LHRH (1-10) - specific antibodies. While contradictory in vitro studies of this kind in the prior art pose interesting questions about the nature of peptide and protein folding, they throw little light on the biological consequence that might ensure in vivo when the LHRH (1-10) hormone molecule is confronted with the choice of binding either to its natural substrate, the LHRH receptor protein, or to an invoked antibody molecule having a specificity that is not primarily directed toward LHRH (1-10) but to a fragment thereof.

In these circumstances where LHRH (1-10) is subject to competitive binding between two possible substrates and where the relative binding affinities to the two substrates may differ in an unknown manner, the new art provides that its particular antibodies cross react with LHRH (1-10) in such a manner that effective concentrations of the hormone are denied to its endogenous receptor and LHRH (1-10) mediated biological processes are blocked.

While in the prior art (USP 4608251) it was known that a particular nonapeptide and a particular decapeptide, each being derived from the single octapeptide LHRH (3-10) could be used to form immunogens that would induce mammals to produce antibodies that react with LHRH (1-10), the surprising discovery of the present invention is the diversity of much smaller peptides that can be used directly as haptens to achieve the same

effect. The recognition of this diversity greatly increases opportunities for the design and manufacture of anti LHRH vaccines and simultaneously provides substantial improvements in the cost: benefit ratio of such vaccines. The diversity extends to peptides that are primarily representative of the N-terminus or of the C terminus or of the mid molecule structure of the parent LHRH (1-10) molecule. Individual anti LHRH vaccines might be formed by using any of the nominated penta, hexa or heptapeptides but generally preferred peptides are those that retain the pyroglutamic acid at the N-terminus or that retain the glycine amide at the C-terminus. A good example of a preferred peptide is the heptapeptide comprising LHRH (1-7). This substance combines the properties of a relatively cost-effective synthesis, ease of conjugation to immunogenic proteins and high biological potency when used as hapten.

The novel immunogens of the invention are formed by chemical conjugation of the designated peptides to any of the immunogenic proteins known in the art, exemplified by but not limited to serum albumins, thyroglobulin, ovalbumin, gelatin, haemocyanin, serum globulin and the like. The chemical processes used to form the immunogenic conjugates may be any of those known in the art to be capable of inducing a covalent bond between the peptide and protein including the use of water-soluble carbodiimide reagents, solvent-soluble carbodiimide reagents particularly in combination with N-hydroxysuccinimide or N-hydroxybenztriazole, and the use of glutaraldehyde or alkyl and aryl diisocyanates. Those skilled in the art of peptide to protein conjugation chemistry will recognise that conjugation to proteins of various of the peptides designated in this invention will be facilitated if they be chemically modified at either end with reagents such as the amino acid cysteine to

introduce thiol functionality or the amino acid lysine to introduce amino functionality. Immunogens formed by the device of firstly adding an extra chemically reactive amino acid, or a group of amino acids terminating in such a reactive amino acid, to either N or the C terminus of the designated peptides and then using conjugation procedures to link such peptides to carrier proteins fall within the scope of the invention. Likewise, designated peptides having a free amino terminus may be linked to thiol-containing proteins by first reacting them with reagents capable of introducing an activated carbon to carbon double bond into the peptide. Such reagents are exemplified by N-succinimidyl-3-maleimido benzoate and immunogenic peptide:protein conjugates formed by this device fall within the scope of the invention. The essential feature of the immunogens claimed herein is that they are proteins bearing the designated peptides as chemically-linked haptens and formed by any of the procedures known in the art. The vaccines of this new art are comprised of any of its novel immunogens used in combination with an immunoadjuvant including typically but not limited to polycationic and polyanionic polyelectrolytes, alhydrogel, mineral oil emulsions or combinations thereof particularly emulsions of a polyelectrolyte and a mineral oil. The vaccination methods of the invention are any of those known in the art to confer anti-hapten immunity on vaccinated animals.

The amino acid sequence of LHRH (1-10) is a highly conserved peptide sequence between classes of mammals and, accordingly, the vaccines of the invention are capable of inducing immunoneutering of male and female animals among a wide class including those that may be farmed for fibre, meat, skin or milk, or among those animals that are widely regarded as companion animals. Whilst, in the first instance dependent upon the formation of anti LHRH (1-10)-

binding antibodies, the biological consequences of immunoneutering livestock are many and diverse and include the prevention of estrous behaviour, prevention of ovulation, suppression or fertility, involution of adult testes, suppression of male libido, prevention or suppression of testicular development in growing prepubertal males as well as the suppression of gonadal sex steroid secretion. It is shown herein that the vaccines of this new art, using its novel immunogens, are capable of achieving all these effects thereby creating an opportunity for a new generation of anti LHRH vaccines with diverse applications. The peptides used in the following examples to illustrate this invention are:

LHRH (1-5) comprising pGlu-His-Trp-Ser-Tyr-OH
LHRH (1-6) comprising pGlu-His-Trp-Ser-Tyr-Gly-OH
LHRH (1-7) comprising pGlu-His-Trp-Ser-Tyr-Gly-Leu-OH
LHRH (2-8) comprising H-His-Trp-Ser-Tyr-Gly-Leu-Arg-OH
LHRH (3-8) comprising H-Trp-Ser-Tyr-Gly-Leu-Arg-OH
LHRH (4-10) comprising H-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2
LHRH (5-10 comprising H-Tyr-Gly-Leu-Arg-Pro-Gly-HN_2

Symbols used herein to represent the immunogenic conjugates of the invention are illustrated, for example, by LHRH (1-5):HSA or LHRH (3-8):BSA wherein the former is intended to represent a chemical conjugate of LHRH (1-5) with human serum albumin and the latter to represent a chemical conjugate of LHRH (3-8) with bovine serum albumin. Those skilled in the art will recognise that such symbols fail to represent the fact that depending on the carrier protein it may be possible to conjugate as little as 1 and as many as about 50 molecules of LHRH-fragment peptide to that carrier protein, the exact number being an expression of the epitope density when the epitope is the LHRH fragment peptide. This invention places no limits on the epitope density to be achieved in the immunogenic conjugates of its methods and processes,

although it is already well known in the art that the epitope density can influence the immunological properties of the conjugate.

To achieve the biological effects of immunoneuting livestock it is necessary to cause them to produce antibodies that bind to LHRH (1-10) and thereby block its biological activity. A common procedure for demonstrating the presence of such antibodies in the sera of immune animals is to perform an in vitro measurement of the anti LHRH (1-10) antibody titre. Such measurements can be used to confirm the presence or absence of antibodies that bind with LHRH (1-10).

It would be expected from the prior art that the novel vaccines of the invention would be cable of provoking anti peptide antibodies when the peptide is any of the peptide fragments designated herein. That property alone would not be expected to achieve the biological effects of immunoneutering because none of the designated peptide fragments are known to possess the biological activity of LHRH (1-10). It is a feature of the invention that the antibody provoked by its vaccines produce, in addition to anti-peptide fragment antibodies, anti LHRH (1-10) antibody titres as well that are frequently of the same order of magnitude as those which can be achieved when LHRH (1-10) itself is used as hapten. Because of this fact the biological effects caused by the novel vaccines can be understood.

In general there will be four major influences that determine the outcome of an immunoneutering vaccination with the vaccines of the invention. These will be: (i) the sex of the target animal; (ii) the species of the target animal; (iii) the particular peptide fragment used to formulate the vaccine; and (iv) the magnitude of the anti LHRH (1-10) antibody titre provoked by the vaccine. The invention recognizes that, to most efficiently achieve

the biological effects of immunoneutering of a given sex, either within or between species, it may be necessary to optimize both the nature of the peptide fragment used in vaccine preparation and the level of anti LHRH (1-10) antibody titre it provokes, both these last two variables being subject to experimentation.

Examples 1-10 illustrate procedures for the formation of the immunogenic conjugates of the new art using a well known water soluble carbodimide reagent to effect chemical condensation of designated peptides with carrier proteins in an entirely aqueous reaction procedure.

Examples 11-13 illustrate procedures for the formation of the immunogenic conjugates of the new art using a solvent-soluble carbodiimide reagent to effect peptide:protein conjugation in a mixed solvent system.

Example 14 describes the method used for the measurement of anti LHRH (1-10) antibody titre in the plasma of vaccinated animals.

Example 15 illustrates two kinds of new art vaccines that are effective in inducing anti LHRH (1-10) antibodies in vaccinated animals.

In Examples 16-19 and Table 1 it is shown that the new art of the invention is effective in inducing anti LHRH (1-10) antibodies and in blocking the oestrous cycles of immuno neutered Merino ewes.

Examples 20 and 21 together with Tables 2-5 demonstrate that the new art in comparison with prior art is effective with adult Merino rams including the induction of anti LHRH (1-10) antibodies, reducing the serving capacity, causing involution of the testes and diminishing the plasma testosterone concentration of immunoneutered rams.

Examples 22-25 together with Tables 6-9 demonstrate that the new art is effective with mice in provoking anti LHRH (1-10) antibodies, in blocking both male and female

fertility and in suppressing plasma testosterone concentration in vaccinated males.

Examples 26-30 and Table 10 demonstrate that the new art is effective with growing immunoneutered crossbred ram lambs in provoking anti LHRH (1-10) antibodies and in suppressing prepubertal testicular development.

Examples 31-33 and Table 11 demonstrate that the new art is effective with immunoneutered, adult, seasonally anoestrous ewes in provoking anti LHRH (1-10) antibodies and in suppressing both the oestrous and ovulatory response that accompanies the sudden introduction or rams into a flock of seasonally anoestrous ewes.

Example 34 and Table 12 demonstrate that with immunoneutered female cattle, the new art is effective in inducing anti LHRH (1-10) antibodies, in suppressing oestrous behaviour and, as can be inferred from the suppressed concentrations of plasma progesterone, it is effective in suppressing ovulatory capability. These examples illustrate the scope of the invention that extends to all mammals whose reproductive activity is regulated by LHRH (1-10).

Throughout the following examples given to illustrate the invention the values of measured anti LHRH (1-10) antibody titre are presented merely to confirm that such antibodies are in fact formed in response to vaccination. Where mean values for experimental variables are presented in the Tables in the form a ± b (a and b being numbers) these values represent treatment group mean values plus or minus the standard error of the mean.

EXAMPLE 1

Preparation of an LHRH (1-5):Human Serum Albumin Conjugate with a Water Soluble Carbodiimide Condensing Reagent

LHRH (1-5) (100mg) was dissolved in deionized water (5 ml) and added to a stirred solution of human serum albumin (HSA, 100 mg) dissolved in d ionized water

(5 ml). To the combined solutions at ambient temperature was added ethyldimethylaminopropyl carbodiimide hydrochloride (1.0 g) which immediately dissolved. The solution was immediately adjusted to pH 6.5 and maintained at pH 6.5 by the metered addition of sodium hydroxide solution (4 Mol l^{-1}) for 5 h. The product was dialysed against 2 litres of deionized water (3 changes per day for 4 days). Finally, lyophilization yielded the immunogenic LHRH (1-5):HSA conjugate.

EXAMPLE 2

An example according to Example 1 wherein LHRH (1-6) was used instead of LHRH (1-5).

EXAMPLE 3

An example according to Example 1 wherein LHRH (1-7) was used instead of LHRH (1-5).

EXAMPLE 4

An example according to Example 1 wherein LHRH (2-8) was used instead of LHRH (1-5).

EXAMPLE 5

An example according to Example 1 wherein LHRH (4-10) was used instead of LHRH (1-5).

EXAMPLE 6

An example according to Example 1 wherein LHRH (5-10) was used instead of LHRH (1-5).

EXAMPLE 7

An example according to Example 5 wherein bovine serum albumin (BSA) was used as carrier protein instead of human serum albumin.

EXAMPLE 8

An example according to Example 3 wherein bovine serum albumin was used instead of human serum albumin.

EXAMPLE 9

An example according to Example 4 wherein bovine serum albumin was used instead of human serum albumin.

EXAMPLE 10

An example according to Example 1 wherein LHRH (3-8) was used instead of LHRH (1-5) and bovine serum albumin was used instead of human serum albumin.

EXAMPLE 11

To LHRH (1-5) (100 mg) was added with stirring a solution of N-hydroxy succinimide (50 mg) in dry dimethylformamide (1.1 ml). To the resulting solution was added a solution of dicyclohexylcarbodiimide (140 mg) in dry dimethylformamide (0.7 ml) and the mixture allowed to stand at ambient temperature for 3 hours. To the solution was then added with stirring and cooling a solution of human serum albumin (HSA, 100 mg) in phosphate buffer (0.05 Mol l^{-1}, pH 7.8, 4 ml). The pH was immediately readjusted to 7.5 with saturated sodium carbonate solution and the mixture kept for 16 hours at ambient temperature. The reaction mixture was then diluted with water, centrifuged to remove insoluble products and the supernatant liquid extensively dialysed against deionized water (10 litres, 3 changes per day) for 4 days and then lyophilized to yield the immunogenic LHRH (1-5):HSA conjugate.

EXAMPLE 12

An example according to Example 11 wherein LHRH (1-6) was used instead of LHRH (1-5) free acid.

EXAMPLE 13

An example according to Example 11 wherein LHRH (1-7) was used instead of (1-5) free acid.

EXAMPLE 14

Measurement of Anti LHRH (1-10) Antibody Titre by Radioimmunoassay

Anti LHRH (1-10) antibody titre was determined in an assay similar to that reported by G.E. Abraham in Acta Endocrinologica, Volume 75, Supplement 183, Pages 7-42 (1974) for steroid specific antibodies. Plasma was

serially diluted with sodium phosphate buffer (0.05 Mol l^{-1} pH 7.4) containing gelatin (0.1%), sodium chloride (0.9%) and sodium azide (0.1%). To 0.1 ml of diluted plasma was added radiolabelled I^{125}-LHRH (1-10) (3.7 TBq/mole, 10000 dpm) in 0.9 ml phosphate buffer. The mixture was kept at 4°C for 16 h after which was added a suspension of dextran-coated charcoal (0.2 ml consisting of 2% (w/v) decolourizing charcoal (Ajax Chemicals Pty. Ltd.) suspended in phosphate buffer containing 0.1% (w/v) dextran T-70 (Pharmacia Pty. Ltd). The solution was allowed to equilibrate for 1 h with the added charcoal to allow adsorption of the free radioligand and was then centrifuged (1000 g, 15 min) to pellet the charcoal. The supernatant solution was removed by suction and the radioactivity on the charcoal pellet measured by gamma counting. The antibody titre is defined as the dilution of antiserum which binds 50% of the I^{125}-LHRH (1-10) available and is expressed as the reciprocal. The between assay coefficient of variation was 16%.

EXAMPLE 15

Type 1 Vaccines

The type 1 vaccines used in the examples given herein were formed by dissolving 1 mg of the immunogenic LHRH fragment:HSA conjugate in physiological saline (1 ml) and emulsifying with Freund's complete adjuvant (1 ml).

Type 2 vaccines

The type 2 vaccines used in the examples given herein were formed by dissolving 1 mg of the immunogenic LHRH fragment:HSA conjugate in 0.6 ml of 20% DEAE-dextran (diethylaminoethyldextran) and emulsifying with 1.4 ml of a solution of the oil-soluble surface active agent Arlacel 80 (1 part v/v) in a mineral oil (5 parts v/v).

In both Type 1 and Type 2 vaccines the LHRH fragments can be comprised of any of those designated LHRH fragment peptides that can be used to make the immunogenic

conjugates of the inv ntion.

EXAMPLE 16

Effect on Anti LHRH (1-10) Antibody Titre and on the Oestrous Cycles of Adult Merino Ewes Following Vaccination Against LHRH (1-10):HSA

The following is an example of prior art, presented to allow a comparison with the new art of the invention. A group of nine adult Merino ewes were vaccinated against LHRH (1-10) using a LHRH (1-10):HSA conjugate in a Type 1 vaccine (Example 15). Vaccination was comprised of a primary treatment given as a 1 ml injection, intramuscularly to each hind leg. A booster vaccination of the same type and by the same route was given 14 weeks subsequently. The flock was maintained at pasture together with a group of 18 randomly selected unvaccinated control ewes.

One week post booster vaccination a blood sample was taken by jugular venepuncture for the measurement of plasma anti LHRH (1-10) antibody titre. At this rime three vasectomised rams fitted with coloured marking crayons were introduced to the flock. Once each week the ewes were examined, as is commonly done in the art, for the occurrence of oestrous "marks" left by the ram that are indicative of the occurrence of oestrous behaviour of the ewe. The colour of the marking crayons was changed weekly and the posterior portion of the ewes regularly clipped to allow unambiguous detection of marks. Antibody responses were measured in the vaccinated ewes and the occurrence of oestrous cycles measured during a 100 day period during the breeding season when unvaccinated control ewes were expressing normal behavioural oestrous.

The immunoneutering effects of this vaccination are recorded in Table 1.

EXAMPLE 17

Effect on Anti LHRH (1-10) Antibody Titre and on the Oestrous Cycles of Adult Merino Ewes Following Vaccination Against LHRH (1-5):HSA

An example according to Example 16 wherein LHRH (1-5):HSA was used as immunogen instead of LHRH (1-10):HSA such that 9 ewes were given an Example 1 type conjugate and 9 others were given and Example 11 type conjugate. The immunoneutering effects of this vaccination recorded in Table 1 confirm that the new art can block oestrous behaviour.

EXAMPLE 18

Effect on Anti LHRH (1-10) Antibody Titre and on the Oestrous Cycles of Adult Merino Ewes Following Vaccination Against LHRH (1-6):HSA

An example according to Example 16 wherein LHRH (1-6):HSA was used as immunogen instead of LHRH (1-10):HSA such that 9 ewes were given an Example 2 type conjugate and 9 others were given an Example 12 type conjugate. The immunoneutering effects of this vaccination recorded in Table 1 confirm that the new art can block oestrous behaviour.

EXAMPLE 19

Effect on Anti LHRH (1-10) Antibody Titre and on the Oestrous Cycles of Adult Merino Ewes Following Vaccination Against LHRH (1-7):HSA

An example according to Example 16 wherein LHRH (1-7):HSA was used as immunogen instead of LHRH (1-10):HSA such that 9 ewes were given Example 3 type conjugate and 9 others were given an Example 13 type conjugate. The immunoneutering effects of this vaccination recorded in Table 1 confirm that the new art can block oestrous behaviour.

Table 1

Antibody response and the incidence of oestrous cycles in control, LHRH-immune and LHRH fragment-immune ewes during a 100 day period during the breeding season

Treatment group	Immunogen type	Mean anti LHRH (1-10) antibody titre	Mean number of oestrus cycles observed
Control	-	-	4.8 ± 0.3
LHRH (1-10) immune	Example 1*	1,000,000	0
LHRH (1-5) immune	Example 1	57,200	0
LHRH (1-5) immune	Example 11	40,200	0
LHRH (1-6) immune	Example 2	205,000	0
LHRH (1-6) immune	Example 12	18,400	0
LHRH (1-7) immune	Example 3	135,000	0
LHRH (1-7) immune	Example 13	67,900	0

* This immunogen was made by the method of Example 1 except that LHRH (1-10) free acid form was used instead of LHRH (1-5).

EXAMPLE 20

Effect on Anti LHRH (1-10) Antibody Titre, Plasma Testosterone Concentration, Testis Size and Serving Capacity of Adult Merino Rams Following Vaccination Against LHRH (1-10):HSA

This is an example of the prior art given to enable a comparison with the new art of the invention.

Four adult Merino rams were vaccinated against LHRH (1-10) using an LHRH (1-10):HSA conjugate in a Type 1 vaccine (Example 15). Vaccination was comprised of a primary treatment given as a 1 ml intramuscular injection to each hind leg. A booster vaccination of the same type and by the same route was given approximately 14 weeks subsequently. The rams were maintained at pasture

together with four randomly selected unvaccinated control rams. One week post booster vaccination a blood sample was taken by jugular venepuncture for the measurement of plasma LHRH (1-10)-specific antibody titre in the boost response. Subsequently, at various intervals, measurements were made of anti LHRH (1-10) antibody titre, testosterone concentration, serving capacity and testis size of all rams in the study. The effect of the prior art immunoneutering vaccination on these variables is shown in Tables 2 to 5.

EXAMPLE 21

Effect on Anti LHRH (1-10) Antibody Titre, Plasma Testosterone Concentration, Testis Size and Serving Capacity of Adult Merino Rams Following Vaccination Against LHRH (1-7):HSA

An example according to Example 20 wherein LHRH (1-7):HSA (Example 3) was used instead of LHRH (1-10):HSA. The effects of this vaccination are given in Tables 2 to 5 and they confirm the immunoneutering capability of the new art.

Table 2

Anti LHRH (1-10) antibody titres in vaccinated adult Merino rams

Day of experiment	Anti LHRH (1-10) antibody titre in indicated treatment group	
	LHRH (1-10) immune	LHRH (1-7) immune
30	289 ± 98	542 ± 44
111	4310 ± 2136	12437 ± 3213
214	730 ± 139	1222 ± 216
340	226 ± 158	834 ± 444

Table 3

Serving capacity* of adult LHRH (1-10) immune, LHRH (1-7) immune and control rams

Day	Mean serving capacity of rams in indicated treatment group		
	LHRH (1-10) immune	LHRH (1-7) immune	Control
0#	7 ± 1.1	6.8 ± 0.8	8.0 ± 1.6
111	7.5 ± 1.8	4.8 ± 0.8	8.5 ± 1.0
125	4.3 ± 0.9	5.3 ± 1.0	6.5 ± 1.0
157	4.0 ± 0.8	3.3 ± 0.8	8.3 ± 2.0
178	2.8 ± 0.9[a]	2.3 ± 0.5[a]	7.0 ± 1.5
214	2.3 ± 0.5[a]	2.5 ± 1.0[a]	6.3 ± 1.1
309	0.5 ± 0.3[b]	1.5 ± 1.0[b]	5.8 ± 0.3

* Mean number of services given to oestrous ewes. Calculated by taking the mean of the combined number of services given during opportunities on each of 2 consecutive days. Measurements were made by the procedure of P.E. Mattner and A.W.H. Braden reported in the Australian Journal of Experiment Agriculture and Animal Husbandry, Volume 15, Page 330 (1975).

\# Primary vaccination given on day 0; booster vaccination given on day 101.

a,b Values in the same row with different superscripts differ; a, $P < 0.05$; b, $P < 0.01$.

Table 4

Testis size* of LHRH (1-10) immune, LHRH (1-7) immune and control rams

Day	Mean testis size of rams in indicated treatment group		
	LHRH (1-10) immune	LHRH (1-7) immune	Control
157#	50 ± 15[a]	38 ± 5[a]	98 ± 3
178	35 ± 7[b]	24 ± 2[b]	100
214	43 ± 8[b]	31 ± 2[b]	100

* Testis size is expressed as a percentage of the pre vaccination volume and estimated by the comparative palpation procedure of C.M. Oldham, N.R. Adams, P.B. Gheradi, D.R. Lindsay and J.B. Mackintosh reported in the Australian Journal of Agriculture Research, Volume 29, Pages 173-9 (1978).

\# Day 157 is 56 days post booster vaccination

a,b Values in rows with different superscripts differ; a, $P < 0.05$; b, $P < 0.01$.

Table 5

Mean testosterone concentration (ng/ml) in the plasma of LHRH (1-10) immune, LHRH (1-7) immune and control rams

Day	Mean testosterone* concentrations (ng/ml) in indicated treatment group		
	LHRH (1-10) immune	LHRH (1-7) immune	Control
111	0.2 ± 0.07[a]	0.08 ± 0.08[a]	10.8 ± 1.8
214	0.2 ± 0.2[a]	0.12 ± 0.07[a]	4.3 ± 1.3

* Day 111 is 10 days post booster vaccination.

Values in the same row with diff rent superscripts differ; ($P < 0.01$).

EXAMPLE 22

Effect on Anti LHRH (1-10) Antibody Titre and the Fertility of Male Quackenbush Mice Following Vaccination Against LHRH (1-10):HSA

This is an example of the prior art given to enable a comparison with the new art of the invention.

Eight male Quackenbush mice having proven fertility were vaccinated against LHRH (1-10):HSA using Type 1 vaccine (0.2 ml; Example 15) intraperitoneally. A booster vaccination of the same type and by the same route was administered 4 weeks subsequently. Two weeks post boost the mice were bled retro occularly to obtain an 0.1 ml plasma sample for the measurement of anti LHRH (1-10) antibody titre. At this time each vaccinated male mouse was boxed together for 6 weeks with a single female of the same strain that had proven fertility. The incidence of pregnancy among the females during this period was observed. Immediately following the 6 week breeding opportunity all females that had failed to conceive were isolated for 7 days and then given a second opportunity (7 days only) to breed with a different non vaccinated control male that had proven fertility. The incidence of pregnancy among the females was again observed. In this manner it was established that the failure of any female to breed with an LHRH (1-10) immune male was directly attributable to male infertility induced by the effects of the vaccination. Thus the statistical comparison that is possible in this study is the incidence of fertility (or infertility) in the group of LHRH (1-10)-immune male mice compared with that of unvaccinated controls when given the opportunity to breed with a single group of proven fertile females.

Throughout the study, water and food were available ad libitum. The anti fertility effects of this vaccination are shown in Table 6.

For the purpose of Examples 22 to 25 the concept of "proven fertility", that is, the ability to breed, means that each fertile female was known to have previously delivered at least three litters and each fertile male was known to have previously sired at least three litters. Fertility (%) in Tables 6-9 means the percentage of animals in a treatment group capable of breeding.

Table 6

Anti LHRH (1-10) antibody titre and fertility of LHRH (1-10) immune and control Quackenbush male mice

Treatment Group	Antibody titre	Fertility (%)
LHRH (1-10) immune	64450 ± 21240	0[a]
Control	0	100

a Significantly different to control ($P < 0.001$).

EXAMPLE 23

Effect on Anti LHRH (1-10) Antibody Titre, Plasma Testosterone Concentration and the Fertility of Male Quackenbush Mice Following Vaccination Against LHRH (1-7):HSA

Seven male Quackenbush mice of previously proven fertility were vaccinated against LHRH (1-7):HSA using Type 1 vaccine (0.2 ml, Example 15) intraperitoneally. A booster vaccination of the same type and given by the same route was administered 4 weeks subsequently. Two weeks post boost the mice were bled retro occularly to obtain a 0.1 ml sample of plasma for the measurement of specific anti LHRH (1-10) antibody titre and of plasma testosterone concentration. At this time each male LHRH-immune mouse

was boxed together with a single female of the same strain and having previously proven fertility, for a period of 6 weeks. The incidence of pregnancy among the females during this period was observed. Immediately following the 6 week breeding opportunity all females that had failed to conceive were isolated for 7 days and then given a secondary opportunity (7 days only) to breed with a different nonvaccinated control male of previously proven fertility. In this manner it was established that the failure to breed with an LHRH (1-10) immune male was directly attributable to male infertility. Throughout the study water and food were available ad libitum. The anti fertility effects of this vaccination are shown in Table 7.

Table 7

Anti LHRH (1-10) antibody titre, plasma testosterone concentration and fertility in LHRH (1-7) immune and control Quackenbush male mice

Treatment group	Antibody titre	Plasma testosterone (ng/ml)	Fertility %
LHRH (1-7) immune	3190 ± 1130	2.3 ± 0.7[a]	0[b]
Control	0	14.2 ± 3.5	100

a Significantly different to controls, $P < 0.02$

b Significantly different to control, $P < 0.001$.

EXAMPLE 24

Effect on Anti LHRH (1-10) Antibody Titre and the Fertility of Female Quackenbush Mice Following Vaccination Against LHRH (1-7):HSA

Seven female Quackenbush mice of previously proven fertility were vaccinated against LHRH (1-7):HSA using a

Type 1 vaccine (0.2 ml, Example 15) intraperitoneally. A booster vaccination of the same type and by the same route was given 4 weeks subsequently. Two weeks post boost the mice were bled retro occularly to obtain a 0.1 ml sample of plasma for the measurement of anti LHRH (1-10) antibody titre. At this time each female LHRH-immune mouse was boxed for 6 weeks together with a single male of the same strain that had previously proven fertility. The incidence of pregnancy among the females in this period was observed and any pregnancy used to confirm the fertility of that female. Three months following the first booster vaccination a second booster vaccination was given to each female and the opportunity to breed with a fertile male repeated as previously. Again any pregnancy among the females was used to confirm the fertility of that female. Throughout the study water and food were available ad libitum. The anti fertility effects of this vaccination are shown in Table 8.

Table 8

Antibody titre and fertility in female Quackenbush mice vaccinated against LHRH (1-7):HSA

Time during treatment	Antibody titre	Fertility (%)
Pre vaccination	-	100
Post first boost vaccination	5126 ± 1783	28.5[a]
Post second boost vaccination	10175 ± 2271	0[a]

a Significantly different to prevaccination fertility ($P < 0.01$)

EXAMPLE 25

Effect on Anti LHRH (1-10) Antibody Titre, Plasma Testosterone Concentration and the Fertility of Male Quackenbush Mice Following Vaccination Against LHRH (1-6):HSA

An example according to Example 23 except that mice were vaccinated against LHRH (1-6):HSA and 16 weeks following the first booster vaccination the vaccinated males were given a second booster vaccination and then given an opportunity to breed with fertile females. The occurrence of pregnancy in any female was taken as proof of the fertility of her male mate. The anti fertility effects of this vaccination are shown in Table 9.

Table 9

Anti LHRH (1-10) antibody titre, plasma testosterone concentration and fertility of LHRH (1-6) immune and control Quackenbush male mice

Variable	Treatment group	
	LHRH (1-6) immune	Control
14 days post first boost vaccination		
Anti LHRH antibody titre	4531 ± 1310	Nil
Testosterone concentration (ng/ml)	1.7 ± 0.86[a]	16.9 ± 4.1
14 days post second boost vaccination		
Anti LHRH antibody titre	3353 ± 923	Nil
Testosterone concentration (ng/ml)	1.5 ± 1.1[a]	15.6 ± 3.6
Male fertility (%)	25[a]	100

a Significantly different to controls, $P < 0.01$.

EXAMPLE 26

Effect on Anti LHRH (1-10) Antibody Production, and Testicular Development in Growing Crossbred Ram Lambs Vaccinated Against LHRH (4-10):BSA

Fifteen crossbred ram lambs, the progeny of Poll Dorset sires and Border Leicester cross Merino dams, were vaccinated against LHRH (4-10) using a LHRH (4-10):BSA conjugate (Example 7) in a Type 2 vaccine formulation (Example 15). A primary vaccination (2 ml) was given subcutaneously in the neck region when the lambs had a mean age of about 3 weeks. Six weeks subsequently a blood sample was taken by jugular venepuncture for the measurement of anti LHRH antibodies. At this time a measurement of testicular volume was made by the palpation procedure. Additionally, a booster vaccination of the same kind as the primary was given in the same volume and by the same route. Seven days following the boost a further blood sample was taken for measurement of the antibody response. Twenty one days following the boost, testicular volume was again measured and the rams were weaned from their mothers. Throughout this study ewe mothers and ram lambs were kept at pasture. Control lambs used to enable statistical comparisons with the foregoing treatment comprised a comparable group of untreated sexually entire ram lambs (N = 30).

Table 10 reports the effect of the vaccination against LHRH (4-10) on anti LHRH (1-1) antibody production and on the retardation of testicular development in these growing crossbreed lambs.

Table 10

Antibody LHRH (1-10) antibody response and testicular volumes in vaccinated crossbred lambs

Variable	Day of Experiment	Treatment group					
		LHRH (4-10)	LHRH (1-7)	LHRH(2-8)	LHRH (3-8)	LHRH (1-10)	Untreated control
Anti LHRH (1-10) antibody titre	42	105 ± 36	2020 ± 560	2300 ± 750	2160 ± 830	1190 ± 220	0
Mean Testicular volume (cubic centimetres)	42	22 ± 3[a]	22 ± 3[a]	35 ± 5[a]	29 ± 6	27 ± 4	48 ± 4
Anti LHRH (1-10 antibody titre	49	2724 ± 580	7200 ± 1100	5568 ± 1530	3800 ± 800	3255 ± 410	0
Mean Testicular volume (cubic centimetres)	63	16 ± 0.7[a]	20 ± 5[a]	61 ± 7[a]	55 + 9[a]	38 + 12[a]	125 ± 17

a Significantly different to controls (P 0.05)

EXAMPLE 27

Effect on Anti LHRH (1-10) Antibody Production and Testicular Development in Growing Crossbred Lambs Vaccinated Against LHRH (1-7):BSA

An example according to Example 26 wherein LHRH (1-7):BSA (Example 8) was used as immunogen instead of LHRH (4-10):BSA. Table 10 reports the anti gonadal effect of this vaccination.

EXAMPLE 28

Effect on Anti LHRH (1-10) Antibody Production and Testicular Development in Growing Crossbred Lambs Vaccinated Against LHRH (2-8):BSA

An example according to Example 26 wherein LHRH (2-8):BSA (Example 9) was used as immunogen instead of LHRH (4-10):BSA. Table 10 reports the anti gonadal effects of this vaccination.

EXAMPLE 29

Effect on Anti LHRH (1-10) Antibody Production and Testicular Development in Growing Crossbred Lambs Vaccinated Against LHRH (3-8):BSA

An example according to Example 26 wherein LHRH (3-8):BSA (Example 10) was used as immunogen instead of LHRH (4-10):BSA. Table 10 reports the anti gonadal effects of this vaccination.

EXAMPLE 30

Effect on Anti LHRH (1-10) Antibody Response and Testicular Development in Growing Crossbred Lambs Vaccinated Against LHRH (1-10):HSA

An example according to Example 26 wherein LHRH (1-10):HSA was used a immunogen instead of LHRH (4-10):BSA. This is an example of the prior art and it is included to allow a comparison with the new art. The anti gonadal effects of this vaccination are recorded in Table 10.

EXAMPLE 31

Effect of Vaccination Against LHRH (5-10):HSA on Anti LHRH Antibody Production and on the Ram-induced Ovulation and Oestrous Responses in Seasonally Anoestrus Merino Ewes

Twenty five adult Merino ewes were isolated from rams by a distance of several kilometres and were vaccinated against LHRH (5-10) using a LHRH (5-10):HSA conjugate (Example 6) in a Type 1 vaccine (Example 15). A primary vaccination (2 ml) was given subcutaneously in the neck region. A booster vaccination of the same type and by the same route was given 7 weeks subsequently. At this time, which was late in the anoestrous season of the ewes, and immediately prior to the boost, a blood sample was taken by jugular venepuncture to allow for the measurement of the primary anti LHRH (1-10) antibody titre. One week following the boost a second blood sample was taken for measurement of the secondary antibody response. At this time 4 vasectomized rams harnessed with marking crayons were introduced to the flock to induce an ovulation response and oestrous behaviour. Eighteen days post-boost the incidence of fresh ovulations in the ewes following introduction of the rams, was determined at laparoscopy. Thirty five days post-boost the flock was examined for "ram marks" indicative of oestrous behaviour in the ewes. Two groups of control ewes used to enable statistical comparisons with the foregoing treatment comprised: (i) a comparable group (N = 25) of Merino ewes vaccinated against LHRH (1-10):HSA with a Type 1 vaccine (Example 15) according to the same protocol and (ii) a group (N = 25) of untreated ewes. Throughout the study all ewes were maintained together at pasture and were kept isolated from rams until these were introduced to the flock as scheduled. The effects of these vaccinations on the suppression of ovulation and of oestrous behaviour are shown in Table 11.

Table 11. Antibody response, incidence of ram-induced oestrous behaviour and ovulation response in control and immunized seasonally anoestrus Merino ewes following introduction of rams

Variable	Day of Experiment#	Treatment group				
		LHRH (5-10) immune	LHRH (4-10) immune	LHRH (2-8) immune	LHRH (1-10) immune	Untreated controls
nti LHRH (1-10) antibody titre	49	5000	2200	15900	14200	Nil
nti LHRH (1-10) antibody titre	56	53400	42800	43230	57800	Nil
Ewes ovulating	67	Nil[a]	Nil[a]	29[a]	Nil[a]	83
estrous behaviour (%) confirmed 5 days post booster vaccination	84	Nil[a]	Nil[a]	24[a]	9[a]	88

Day 1 is regarded as the day of primary vaccination

Significantly different to controls ($P < 0.001$)

EXAMPLE 32

Effect of Vaccination Against LHRH (2-8):HSA on Anti LHRH (1-10) Antibody Production and on the Ram-Induced Oestrus and Ovulatory Response in Seasonally Anoestrus Merino Ewes

An example according to Example 31 wherein the LHRH (2-8):HSA (Example 4) was used as immunogen instead of LHRH (5-10):HSA. The oestrus-suppressing and ovulation-suppressing effect of this vaccination is reported in Table 11.

EXAMPLE 33

Effect of Vaccination Against LHRH (4-10):HSA on Anti LHRH (1-10) Antibody Production and on the Ram-Induced Oestrus and Ovulatory Response in Seasonally Anoestrus Merino Ewes

An example according to Example 31 wherein the LHRH (4-10):HSA (Example 5) was used as immunogen instead of LHRH (5-10):HSA. The oestrus-suppressing and ovulation-suppressing effect of this vaccination is shown is Table 11.

EXAMPLE 34

Effect of Vaccination Against LHRH (1-7):HSA on the Anti LHRH (1-10) Antibody Response, Oestrous Behaviour and Progesterone Concentrations in Crossbred Heifers

Nine crossbred heifers were vaccinated against LHRH (1-7) using an immunogenic conjugate of LHRH (1-7):HSA (Example 3) in a Type 2 vaccine formulation (Example 15). A primary vaccination of 5 ml was administered subcutaneously at one site either side of the neck. Seven weeks subsequently a blood sample was taken by jugular venepuncture for the measurement of the primary antibody response. At this time a booster vaccination of the same type and volume as the primary was administered by the same route as the primary. In addition in intramuscular

injection of a synthetic prostaglandin was given to each heifer to synchronize oestrous cycles of the herd (Estrumate, 1200 i.u.). Twelve days post boost a further blood sample was taken for measurement of the booster antibody response. Additionally a second administration of the synthetic prostaglandin (1200 i.u., intramuscular) was given and KaMarr heat mount detection pads attached to the back of each heifer in the manner than is well known in the art. Nineteen days post boost the incidence of oestrus among the heifers was recorded. Throughout this study a group of 8 control heifers were managed identically except that they remained unvaccinated. All cattle were kept at pasture. The anti-reproductive effects of this vaccination shown in Table 12 are seen to be the significant reduction in oestrous behaviour that is also accompanied by a significant reduction in mean plasma progesterone concentratiopn in the immune heifers compared to controls.

Table 12

Anti LHRH antibody response, oestrous behaviour and progesterone concentration in heifers

Variable	Day of experiment	Treatment group	
		LHRH (1-7) immune	Untreated control
Anti LHRH antibody titre	42	Nil	Nil
Anti LHRH antibody titre	49	301 ± 146	Nil
% Oestrus heifers confirmed confirmed by KaMarr detection	61	13[a]	75
Mean plasma progesterone concentration (ng/ml)	57	0.2 ± 0.1	0.1 ± 0.06
Mean plasma progesterone concentration (ng/ml)	67	0.6 ± 0.4[b]	2.9 ± 0.8

a Significantly different to untreated control ($P < 0.01$)

b Significantly different to untreated control ($P < 0.05$)

CLAIMS

1. A composition for use in the immunoneutering of mammalian animals comprising a peptide conjugated with an immunogenic protein, the composition being characterized in that the peptide is a sequence selected from the group comprising any contiguous 5, 6 or 7 amino acid fragment of the decapeptide pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-GlyNH_2.

2. A composition as claimed in claim 1 in which the peptide includes the pyroglutamic acid from the N-terminus or the glycine amide from the C-terminus of the decapeptide.

3. A composition as claimed in claim 1 in which the peptide includes at its end proximal to the immunogenic protein an additional amino acid or sequence of amino acids such that the resultant peptide is not homologous with the decapeptide.

4. A composition as claimed in claim 3 in which the additional amino acid is a chemically reactive amino acid at either the N-terminus or the C-terminus of the defined peptide sequence.

5. A composition as claimed in any one of claim 1 to 4 in which the peptide is selected from the group comprising

pGlu-His-Trp-Ser-Tyr-OH

pGlu-His-Trp-Ser-Tyr-Gly-OH

pGlu-His-Trp-Ser-Tyr-Gly-Leu-OH

H-His-Trp-Ser-Tyr-Gly-Leu-Arg-OH

H-Trp-Ser-Tyr-Gly-Leu-Arg-OH

H-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2

H-Tyr-Gly-Leu-Arg-Pro-Gly-HN_2

6. A composition as claimed in any one of claims 1 to 5 in which the immunogenic protein is selected from the group comprising serum albumin, thyroglobulin, ovalbumin, gelatin, haemocyanin and serum globulin.

7. A composition as claimed in claim 1 in which the

peptide is selected from the group pGlu-His-Trp-Ser-Tyr-Gly-Leu-OH and H-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2 and the protein is selected from the group comprising bovine serum albumin, human serum albumin, ovalbumin and serum globulin.

8. A vaccine for immunoneutering mammalian animals comprising at least one composition as claimed in any one of claims 1 to 7 and an immunoadjuvant.

9. A vaccine as claimed in claim 8 in which the immunoadjuvant is selected from the group comprising a diethylaminoethyl dextran or another polycationic polyelectrolyte, a polyanionic polyelectrolyte, a mineral oil, or an emulsion of a polycationic or polyanionic polyelectrolyte and a mineral oil.

10. A method for the immunoneutering of male or female mammalian animals comprising administering to the animal an effective amount of a vaccine according to claim 8 or Claim 9.

11. An immunogenic peptide:protein conjugate as herein described with reference to any one of Examples 1 to 13; a vaccine for immunoneutering mammalian animals as herein described with reference to Example 15; or a method for the immunoneutering of mammalian animals comprising administering to the animal a composition according to claim 1 as herein described with reference to Example 15; or a method for the immunoneutering of mammalian animals comprising administering to the animal a composition according to claim 1 as herein described with reference to any one of Examples 16 to 34.

INTERNATIONAL SEARCH REPORT

International Application No PCT/AU 87/00448

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)

According to International Patent Classification (IPC) or to both National Classification and IPC

Int. Cl.[4] A61K 39/385, C07K 7/06

II. FIELDS SEARCHED

Minimum Documentation Searched

Classification System	Classification Symbols
IPC	A61K 39/385

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched

AU : IPC as above; Australian Classification 87.11:10 (SF 114)

III. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
E X	AU,A, 76423/87 (THE STATE OF VICTORIA) 14 January 1988 (14.01.88) See page 5 lines 14-17, and 29-33 and page 7 lines 15-21	(1-9)
X	AU,A, 34497/84 (AKZO N.V.) 26 April 1985 (26.04.85) See example 1	(1-9,11)
X	US,A, 4608251 (Abdus S. MIA) 26 August 1986 (26.08.86)	(1-9,11)
A	AU,B, 80826/75 (503647) (ALL INDIA INSTITUTE OF MEDICAL SCIENCE) 11 November 1976 (11.11.76)	(1-9,11)
A	Chemical Abstracts, Volume 98, No.17, issued 1983, April 25 (Columbus, Ohio, U.S.A.), Donna L. Vogel et al, 'Sertoli cell maturation is impaired by neonatal passive immunization with antiserum to LHRH', abstract No. 137896q	(1-9)

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search

30 March 1988 (30.03.88)

Date of Mailing of this International Search Report

(11.04.88) 11 APRIL 1988

Signature of Authorized Officer

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. [X] Claim numbers 10 ... because they relate to subject matter not required to be searched by this Authority, namely:

Method of treatment of the animal body by therapy.

2. [ ] Claim numbers ..... because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically

3. [ ] Claim numbers..... because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this international application as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. [ ] As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON
INTERNATIONAL APPLICATION NO. PCT/AU 87/00448

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report	Patent Family Members		
AU 34497/84	AU 34498/84	EP 142192	EP 142193
	JP 60172932	JP 60172933	
AU 80826/75	AR 217615	CA 1054937	CH 614626
	DE 2518546	DK 2069/75	FI 751746
	GB 1492445	IL 47177	NL 7505412
	NO 751680	NZ 177422	SE 7505411
	US 4161519	IN 140168	
US 4608251	EP 181236		

END OF ANNEX